UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

75801-2

MEMORANDUM

SUBJECT: Ecological Risk Assessment for *Pseudomonas chlororaphis* Strain 63-28 (PC Code: 006478, AtEze™), a New Microbial Active Ingredient for **FIFRA** Section 3 Registration; DP Barcode: D254493; Case:062551; Submission: S557025

FROM: Gail S. Tomimatsu, Ph. D., Plant Pathologist
Biopesticides and Pollution Prevention Division

THRU: Zigfridas Vaituzis, Ph.D., Senior Scientist
Biopesticides and Pollution Prevention Division

TO: Edward Allen, Regulatory Action Leader
Biopesticides and Pollution Prevention Division

ACTION REQUESTED

To review a request from Agrium Inc., for the Section 3 registration of a new active microbial pesticide, AtEze™ (*Pseudomonas chlororaphis* Strain 63-28, synonym=*Ps. aureofaciens*, PC Code: 006478), intended for use in greenhouses on vegetable and ornamental crops. This risk assessment is based on the submitted materials: 1) Volume 13: Nontarget Organisms: Request to Waive Data Requirements (No MRID #), and 2) Excerpts from Volume 2, MRID #446604-01: "**AtEze™** - Product Identity and Disclosure of Ingredients" . Also, a DER for MRID# 45262-10 (a Product Performance Study entitled, "AtEze™ - Suppression of Diseases Caused by *Rhizoctonia solani* and *Pythium* spp. On Ornamental and Vegetable Crops") is provided, but is not considered as part of the ecological risk assessment.

CONCLUSIONS AND RECOMMENDATIONS

Sufficient information and data regarding ecological effects and environmental fate were submitted to support conditional registration of *Ps. chlororaphis* Strain 63-28, limited to soil-drenches or chemigation of vegetables and ornamentals in commercial greenhouses only.

CONCURRENCES								
SYMBOL	7511C	7511C						
SURNAME	TOMIMATSU	S.A TMUZIS						
DATE	9.22.99	9/22/99						

Additional studies and data for a more comprehensive risk assessment and product characterization must be submitted: (1) growth and survival studies of *Ps. chlororaphis* Strain 63-28 for the temperature range of 10 to 35°C; and (2) data verifying production of siderophores by this strain.

Label use directions should be limited to directed applications or soil drench of contained plants for production crop use only, and the environmental hazards statement should include a statement that product may be toxic/pathogenic to aquatic fish and/or invertebrates.

~~CONTAINS FIFRA-SENSITIVE CONFIDENTIAL BUSINESS INFORMATION~~

BACKGROUND AND ANALYSIS

The applicant, Agrium Inc. is seeking Section 3 registration for viable *Pseudomonas chlororaphis* (synonym: *Ps. aureofaciens*), for intended uses as a soil drench, or through drip irrigation (e.g., chemigation) to suppress stem and root rots caused by *Rhizoctonia solani* and by *Pythium* spp. in greenhouses. Data and text concerning the biology, taxonomy and environmental fate of the MPCA were included in the volume characterizing its identity and biological properties (MRID #446604-01). Waivers from testing the toxicity/infectivity of the MPCA to nontarget organisms were requested in Volume 13 (no MRID #).

Biology and Ecology of *Pseudomonas chlororaphis*

Pseudomonas chlororaphis Strain 63-28 was isolated from roots of a healthy canola plant in a field near Winnipeg, Manitoba, Canada in June 1984. This strain has been tested in commercial greenhouses across Canada in selected ecozones; and in field trials under research permits (from Agriculture Canada and Health Canada: Nos. 132-RP-92 and 112-RP-96). Requirements for crop destruction were waived each time, reportedly due to low risks associated with this strain. Trials were also conducted in commercial greenhouses in California under state permits 608015 and 708005 (1996 and 1997), and in Florida and Ohio (respective state approval in 1997).

One of the most commonly-occurring bacteria in soil and on the roots of many plants (e.g., citrus, wheat, and other field crops), the species is characterized as metabolically versatile, frequently psychrophilic and saprophytic. *Pseudomonas chlororaphis* is often an important component of the pseudomonad community; indigenous population levels of pseudomonads are estimated to be between 10E5 and 10E7 CFU/g soil, depending on the isolation medium.

There are no reports of pathogenicity or toxicity of *Ps. chlororaphis* (or *Ps. aureofaciens*) to humans, wild mammals, or plants. However, pathogenicity of a strain of *Ps. chlororaphis* to salmon fry (*Oncorhynchus rhodurus*) was reported in Japan (Hatai, et al. 1975). This particular strain reportedly was able to kill trout, carp and eel **when the fish were inoculated** (Egusa, 1992). A strain of *Ps. chlororaphis* reportedly caused basal soft rot and discoloration of straw mushrooms in Puerto Rico. One study implied that a strain of *Ps. aureofaciens (=Ps. chlororaphis)* might be implicated in infection of chickens, based upon selective isolation and subsequent identification of bacterial colonies of diseased tissues from dead embryos and baby chicks (Shahata, et al., 1988). *Pseudomonas chlororaphis* has also been isolated from dead

strain of cockchafer (beetle) (Pellerin, 1994); and a strain of *Ps. chlororaphis* was observed to increase the growth of shiitake mushrooms (Raaska and Matila-Sandholm, 1991).

Strains of *Ps. chlororaphis* applied into natural soils do not persist for a long period of time (Kluepfel et al., 1991), nor do they change microbial processes significantly in soils (England, et al., 1993). The growth of *Ps. chlororaphis* in the spermosphere of seed-inoculated sugarbeets exhibited lag phases of 8 to 12 hrs; its population increased mainly between 12 and 24 hrs (Fukui et al., 1994). When introduced at a concentration of approximately 10E6 CFU/g of root, several strains of *P. chlororaphis* fall below detection levels after 8 to 12 weeks (no citation). Available evidence suggests that application of this bacterium to natural soils will have a minimal negative effect on soil beneficial organisms such as *Rhizobium* and mycorrhizal fungi (England et al., 1993; Paulitz and Linderman, 1989).

The strain was reported to be generally suppressive to a number of different species of plant pathogens. It is not considered parasitic; however, it produces a number of antibiotics (e.g., phenazine) and other fungistatic compounds. This strain was also noted to produce siderophores, which sequester ferric iron, an essential element for growth of many fungal pathogens.

In vitro strain 63-28 was also found to produce cytokinins (~ 565 pmoles dihydrozeatin riboside; 5-48 pmoles transzeatin riboside per 0.1 mL supernatant); measured from a modification of Phytodetek™.

Environmental Fate of *Pseudomonas chlororaphis* Strain 63-28 after Application in Greenhouses

This brief study was conducted in order to understand the ability of this strain to survive in the environment after application. Representative plants included *Euphorbia pulcherrima* (poinsettia), *Chrysanthemum mortifolium* (chrysanthemum), *Vinca rosea* (vinca), and *Lycopersicon esculentum* (tomato). The environmental fate of a rifampicin-resistant mutant of the strain was monitored at intervals of 1-4 weeks in growth media, leached water, and root surfaces of plants grown in research or commercial greenhouses. Bacterial suspensions were applied immediately after transplanting, also as soil drenches at the rate of log 9.0 CFU/L of growth medium (Allegro bags, Premier Peat). Tomato fruits also were tested for dispersal of the bacterial strain from the growth medium.

Generally, populations of the rifampicin-resistant *Ps. chlororaphis* Strain 63-28 decreased gradually after application, however the rate of decline varied slightly with different plants or growth media tested. The study author reported that bacterial populations appeared to be more persistent and detectable on roots of poinsettia (after 19 weeks); populations were also recovered from roots of chrysanthemum, vinca, and tomato, however the decline was more dramatic. Bacterial populations were also recovered from leached water, and dropped to "[n]on-detectable levels after 16 weeks, for the ornamentals. Lower bacterial populations were recovered from leached water of tomatoes, then after 8 weeks, became "[n]ondetectable." The author reported that the bacterium was never detected on tomato fruits during the period of the study. No details were provided regarding materials and methodology, statistical procedures or other observations for this study.

The applicant requested waivers from testing of *Ps. chlororaphis* Strain 63-28 on nontarget aquatic freshwater aquatic organisms, honeybees and insects.

Guidelines 885.4050 and 885.4100: Avian Oral Toxicity/Pathogenicity and Respiratory Pathogenicity Tests

Waivers from avian testing through the oral and respiratory routes of exposure are based on: (1) use pattern of soil drench or chemigation in production greenhouses only, as a minimal exposure scenario; (2) results of submitted data verified that *Ps. chlororaphis* 63-28 is unable to grow or even survive at 41 °C; and (3) no adverse effects reported for *Ps. chlororaphis* on avian species from the open literature.

The submitted rationale is sufficient to waive nontarget avian studies for the intended greenhouse uses of *Ps. chlororaphis* 63-28. Also, limited data were submitted to indicate lack of recovery of *Ps. chlororaphis* from tomato fruit following applications; further eliminating the necessity of avian pathogenicity testing via the oral route of exposure. However, if additional use patterns for this product increase exposures to avian species, toxicity and pathogenicity testing may be required.

Guidelines 885.4200 and 882.4240: Freshwater Fish and Aquatic Invertebrate Toxicity and Pathogenicity Tests

The applicant claims that the possibility of runoff water from MPCA-treated greenhouse plants will reach surface water streams, or groundwater reservoirs is extremely low; if instructions on the label are followed, and if suggested volumes of drench can be mostly retained in containers.

The term "greenhouse use" is ambiguous and problematic in U.S. agriculture. Greenhouse structures may be very well-contained glasshouses, spanning over 50 acres for some commercial operations or, they may be open, "lathe" houses; with 2 to 50 acres for nurseries. In view of the wide range of potential exposures of the MPCA to surface or groundwater runoff from US greenhouses or nurseries, especially following chemigation, and because populations of *Ps. chlororaphis* Strain 63-28 have been documented to survive for up to16 weeks, BPPD asserts that some nontarget organisms are likely to be exposed. Exposures are likely to be less dramatic if intended uses of the MPCA are limited to soil drenches for contained plants, and if "runoff" can be adequately contained. The manufacturer claims that "[r]unoff during product application will be minimal if instructions on the label are followed, and suggested volumes of drench can mostly be retained in containers"...runoff water ...normally percolates through soils underneath the greenhouse. Commercial greenhouses also are likely to use automatic watering systems, trough irrigation, spaghetti tubes and drip irrigation, so that watering crops "thoroughly to runoff" no longer seems necessary.

The submitted waiver rationale is minimally satisfactory for fulfillment of this data requirement. BPPD cannot characterize the risks to aquatic organisms in the absence of exposure data. Preliminary data regarding fate submitted by the applicant indicated recovery of

Ps. chlororaphis populations 2-3 months post-application. It is highly likely that the majority of the bacteria will eventually drain or percolate into soil beneath or around greenhouses or nurseries. Although pathogenic risks to nontarget fish (e.g., salmon fry and possibly trout, carp and eel) have been identified, there is no reasonable justification to require nontarget aquatic organism testing for this limited use; and in consideration of other inferential information concerning product characterization. Specifically, the applicant has stated that the ..."[b]acterium is quickly confined to the root zone"... (MRID # 446604-01); typical of many other pseudomonads. Although no data were submitted to support this observation, *Ps. chlororaphis* Strain 63-28 produces siderophores to help sequester iron; *Pseudomonas* spp. which produce siderophores are predominantly confined to root zones.

Label use directions should be limited to directed applications or soil drench of contained plants for production crop use only, and the environmental hazards statement should include a statement that product may be toxic/pathogenic to aquatic fish and/or invertebrates. If additional use patterns for this product increase exposures to aquatic organisms, or if mitigating label language is not used, toxicity and pathogenicity testing will be required.

Guidelines 885.4340 and 885.4380: Toxicity Studies on Non-target Insects and Honey Bees

The applicant is requesting waivers from testing the toxicity of the MPCA to beneficial nontarget insect species and honeybees based on the rationale that the uses will be mainly in enclosed environment (greenhouses), and there was no evidence from the open literature to suggest that *Ps. chlororaphis* or other closely-related species were toxic/pathogenic to living insects.

Because intended uses involve directed applications as a soil drench, or in chemigation systems in enclosed environments, the possibility of exposing the applied bacterium to a natural insect population is extremely low. Potential exposure of released beneficial insects (e.g., to the MPCA) is also likely to be minimal, in consideration of the intended use patterns. Although BPPD cannot characterize or assess the risks of *Ps. chlororaphis* 63-28 without toxicity/pathogenicity data, it appears unlikely that there would be irreversible adverse effects to beneficial insect releases resulting from unintended exposures, based upon limited data regarding the ecology and fate of *Ps. chlororaphis* Strain 63-28.

Therefore, the requirements for testing the toxicity/pathogenicity to nontarget insects and honeybees are waived for this limited intended use of *Ps. chlororaphis* Strain 63-28.

SUMMARY AND CONCLUSIONS

Data concerning the impact of temperature on growth of *Ps. chlororaphis*, Strain 63-28 suggested that optimal temperatures for growth and reproduction were in the range of 25 to 37 °C; although bacteria proliferated more slowly and the final population was significantly lower in comparison to cultures at lower temperatures. The bacteria stopped growing at 41 °C (No MRID #), however no data or information were submitted to determine if *Ps. chlororaphis* Strain 63-28 can survive at temperatures < 25°C. BPPD requests that data (growth and survival) be submitted for the temperature range of 10 to 25 °C; such data will be useful in a more comprehensive risk assessment, particularly since *Ps. chlororaphis* was reported to be "frequently

psychrophilic."

Waiver rationale submitted in lieu of nontarget toxicity/pathogenicity testing are justified for mini mammalian and honeybee exposures for the intended enclosed greenhouse uses. Nontarget aquatic organisms (fish and aquatic invertebrate) and insect studies are also waived for this limited use based upon presumed minimal to no exposure, and limited information regarding ecology of the intended MPCA.

Sufficient information and data regarding ecological effects and environmental fate have been submitted to justify conditional registration of *Ps. chlororaphis* Strain 63-28, limited to soil-drenches or chemigation of vegetables and ornamentals in **commercial greenhouses only.** Additional studies and data regarding the product identity and characterization must be submitted for review for Section 3 registration: (1) *Ps. chlororaphis* Strain 63-28 survival at temperatures < 25° C; and (2) siderophore production.

DATA EVALUATION RECORD

Primary Reviewer: Gail S. Tomimatsu, Ph.D., Plant Pathologist
Secondary Reviewer: Zigfridas Vaituzis, Ph.D., Senior Scientist 9/21/99

MRID No.: ~~45262-10~~ 44626210

Title of Study: AtEze™ - Suppression of Diseases Caused by *Rhizoctonia solani* and *Pythium* spp. On Ornamental and Vegetable Crops

Authors: N. Seresinhe, G. Peng, and G. L. Brown

Study Completed: March, 1998

Sponsor: Agrium US, Inc.
4582 S. Ulster St., Suite 1400
Denver, CO 80237

Performing Laboratories/Greenhouses:
Agrium, Inc., Saskatoon, SK, Canada S7N 2X8
Pest Management Research Station, Agriculture and Agri-Food Canada, Vineland, ON, Canada L0R 2E0
Chase Research Garden, Mt. Aukum, CA 95656-0168
Gulf Coast Research Center, Univ. FL, Bradenton, FL 34203-9324
Plant Pathology Dept., Univ.FL, Gainesville, FL 32611-0680
Pest Management Center, Dept. Biol. Sci., Simon Fraser Univ., Burnaby, BC, Canada V5A 1S6
Premier Research Center. Riviere-du-Loup, Quebec, Canada G5R 3Z1

GLP Compliance Statement:
The reported studies were performed at several research facilities following standard research procedures of microbiology, plant pathology, and crop production. Even though GLP certification was not implemented at reporting facilities, studies were designed and conducted according to standard research procedures: methodologies were described in study protocols, detailed records were taken during experiments, and procedures and results of each experiment were reviewed by the study director.

Study Director: Gary Peng, Ph. D., Agrium Inc.

Company Agent: Gerald L. Brown, Ph.D.

STUDY AUTHOR'S SUMMARY and CONCLUSIONS: The efficacy of AtEze™ (*Pseudomonas chlororaphis*; synonym = *Ps. aureofaciens*) to suppress plant-pathogenic *Rhizoctonia solani* and *Pythium ultimum* on poinsettia, impatiens, cucumbers and tomatoes was demonstrated in several trials, in varying geographic US locations in small research trials (field and greenhouse) and in commercial greenhouses.

REVIEWER'S CONCLUSIONS: The product, At-Eze™ showed some efficacy in suppressing root disease problems of greenhouse-grown ornamentals and vegetables. In some cases, the product enhanced plant vigor, crop quality and crop yields.

CLASSIFICATION OF STUDY: Supplemental.

PURPOSE:

To determine the effectiveness of AtEze™, (a bacterial inoculant of 1.15% *Pseudomonas chlororaphis*, Strain 63-28) intended for the suppression of soil-borne diseases caused by the fungi, *Rhizoctonia solani* and *Pythium ultimum* on greenhouse ornamental and vegetable crops under laboratory and commercial greenhouse conditions.

In this data volume, there were many studies and preliminary experiments which examined production of volatile antifungal substances; and laboratory and research greenhouse trials to determine appropriate efficacy tests on a larger scale. Results of all studies are summarized in the section, "Study Author's Results and Conclusions"; other information and methodologies concerning "efficacy" testing under commercial conditions are detailed below.

BASIC MATERIALS AND METHODS:

1. Research Greenhouse Experiments:

All research greenhouse experiments utilized pathogen- infested (either laboratory-grown *R. solani* or *P. ultimum*) peat and/or soil-less mixes. Crops studied included poinsettia, impatiens, other nursery ornamental seedlings, cucumber (container and hydroponic system) and tomatoes.

Rooted cuttings of poinsettia or seedlings of impatiens were transplanted into growth media containing 75 to 1500 propagules of *R. solani*/mL of media. Immediately after transplanting, diluted inoculant containing approximately $2x10^7$ CFU/mL was applied as a soil drench at a rate of 200 mL per 6-in pot; disease damage was evaluated based on the incidence of stem lesions and degree of plant wilting.

2. Commercial Greenhouse Experiments

a. Individual commercial trials on poinsettias were conducted in 6 greenhouses in Canada (3), California (1), Ohio (1), and Florida (1) intermittently during 1995 through 1997. Rooted cuttings were transplanted into a peat-based potting mix; the bacterial inoculant (AtEze®, $1x10^9$ CFU/ml) was diluted at 1:500 with potable water and applied as a soil drench. Alternative chemical and biological fungicides were applied at recommended rates for comparison. Each treatment consisted of 20 - 25 plants; in some cases, each plant was a replicate and in others, each replicate consisted of 5 plants. Experiments were laid out as a randomized block design at different locations of a greenhouse. No pathogen inoculum was applied artificially; consequently, diseases were caused by pathogens introduced through normal pathways in commercial production (growth media, infested or infected plant materials, or contaminated soil).

b. Commercial rooted cuttings of New Guinea impatiens were planted into 4-in pots, containing a commercial potting mix. AtEze was diluted 1:500 (final density: ~ 2 X 10E6 bacteria/mL: these units are inconsistent with other reported methodology-uncertain if authors meant CFU instead of bacterial cells). The plant height was measured 2 months after the transplant. Non-treated plants were used as controls that were grown as in normal production practices.

c. Seeds of cucumber cv. Corona or Flamingo were planted in a germination mix, and drenched with AtEze (~10E9 CFU/mL), diluted by 1000X with potable water. Seedlings at the 2 - 3 leaf stage were transplanted into soil beds; each plant was drenched again with 150 - 250 mL of product diluted by 250X (no final bacterial population reported). Incidence of naturally-occuring Pythium root rot was monitored over the crop season; yields were taken from treated and non-treated plants, at intervals of 2 -3 days, for 9 to 10 weeks. Yield data were collected as averages of treated or non-treated plots instead of individual plants.

d. Seven trials were conducted in 4 Canadian greenhouses, using different growing systems during 1992 and 1996.

STUDY AUTHOR'S RESULTS AND CONCLUSIONS

Treatments with the bacterial inoculant reduced disease severity on poinsettia by 51 to 74%, and on impatiens by 44 to 67% in comparison to the damage on pathogen controls. When applied at the same rate to potting mix infested with *P. ultimum*, immediately after transplant, the inoculant reduced severity of damage due to root rot by 55-80% on poinsettia and impatiens in comparison to non-treated, pathogen-inoculated plants. With cucumber, the inoculant was applied to pathogen-infested germination medium prior to seeding. The treatment suppressed seed decay and damping off caused by several species of *Pythium*, with disease-control efficacy ranging from 66 to 100%. In two trials where *P. ultimum* caused about 50% stand loss, the inoculant suppressed the disease completely. In a semi-hydroponic growing system, cucumber plants treated with the product yielded 7.1 - 17.4% more fruit in comparison to control plants. In trials where commonly-used fungicides were included for comparison, the inoculant was generally as effective (or more) in suppressing diseases caused by *R. solani* and *Pythium* spp. on the tested crops.

The product enhanced plant vigor and quality of poinsettia as effectively as did conventional chemical fungicides; and in several cases, the product was significantly more effective than fungicides in maintaining plant root health and crop quality. On greenhouse vegetables, a trend of increased yield was observed under various commercial-growing conditions.

AGENCY REVIEWER'S CONCLUSIONS:

The product, At-Eze™ showed some efficacy in suppressing root disease problems of greenhouse-grown ornamentals and vegetables. In some cases, the product enhanced plant vigor, crop quality and crop yields.

LIMITATIONS OF ANALYSIS: The reported results show some efficacy in disease reduction through soil drenches of At-Eze™. Clearly, in some cases, the product may act as a growth regulator, instead of a pesticide. Indeed, as documented in another report (MRID # 446604-01), this strain of *Ps. chlororaphis* (Strain 63-28) was characterized as producing cytokinins, as well as antibiotics and siderophores.

13544

R144085

Chemical: Pseudomonas chlororaphis strain 63-28

PC Code:
006478
HED File Code: 41300 BPPD Eco Effects
Memo Date: 9/22/1999
File ID: DPD254493
Accession #: 000-00-9002

HED Records Reference Center
5/2/2007